Non-muscle myosin II heavy chain has a cryptic cell-adhesion domain

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We have discovered a cryptic cell-adhesion domain in non-muscle myosin II heavy chain. A 205 kDa cell-adhesion-promoting polypeptide (p205) was extracted from BHK cells by Nonidet P-40 or Dounce homogenization. Adhesion to p205 was specifically inhibited by the peptide Gly-Arg-Gly-Asp-Ser-Pro, indicating a role for the Arg-Gly-Asp cell-adhesion motif. Purified p205 was identified as non-muscle myosin II heavy

chain, based on sequence analysis and on the cross-reactivity of p205 with anti-(bovine trachea myosin) antibodies. Further experiments showed that the heavy chain of purified myosin II has cell-adhesion-promoting activity in a cell-blotting assay, and cross-reacted with anti-p205 antibodies. Finally, the adhesion domain was located in the tail portion of myosin II heavy chain, where an Arg-Gly-Asp-containing sequence can be found.

INTRODUCTION

Adhesion-promoting polypeptides can be detected by SDS/PAGE followed by cell blotting. In this procedure, which is analogous to immunoblotting, cells are incubated on nitrocellulose paper, to which polypeptides were previously transferred. Those polypeptides with adhesion-promoting activity will be detected as bands of cells attached to the nitrocellulose. First used in studies aimed at distinguishing plasma fibronectin from vitronectin [1], cell blotting has subsequently been employed for a variety of other applications, including identification of adhesion proteins extracted from the extracellular matrix of bone [2] and synovium [3], analysis of biologically active fragments of thrombospondin [4], and characterization of adhesion-promoting polypeptides in chronic wound fluid and burn fluid [5,6]. This method also has been used to detect molecules that function in bacterial [7] and cell-cell [8] adhesion.

In the present experiments, we used cell blotting to identify fibroblast proteins with adhesion-promoting sequences. Baby-hamster kidney (BHK) cells contained several cell-adhesion-promoting polypeptides, the most prominent of which, p205, was of molecular mass 205 kDa. Surprisingly, this polypeptide turned out to be myosin II heavy chain. Details are reported herein.

MATERIALS AND METHODS

Materials

BHK cells adapted for growth in suspension culture were cultured as described previously [5]. Human plasma fibronectin was obtained from the New York Blood Center, New York, NY, U.S.A. Pig platelet myosin II, bovine adrenal-medulla myosin II, and head and tail domains of adrenal medulla myosin II prepared by papain digestion [9], were generously given by Dr. J. Albanesi, UT Southwestern Medical Center. Rabbit antiserum against bovine trachea myosin II was a gift from Dr. K. Kamm, UT Southwestern Medical Center. Synthetic peptides Gly-Arg-Gly-Asp-Ser-Pro and Gly-Arg-Gly-Glu-Ser-Pro were purchased from Telios Pharmaceuticals (San Diego, CA, U.S.A.).

Cell extraction

BHK cells were extracted by incubating them in Dulbecco's phosphate-buffered saline (DPBS) (150 mM NaCl, 3 mM KCl,

1 mM CaCl₂, 0.5 mM MgCl₂, 1 mM KH₂PO₄, 6 mM Na₂HPO₄, pH 7.2) containing 0.5 % Nonidet P-40 (NP-40) and proteinase inhibitors (0.2 mM phenylmethanesulphonyl fluoride, 1 µg/ml leupeptin, 1 µg/ml pepstatin A) for 5 min at 22 °C. Alternatively, cells were suspended in 10 mM Tris, pH 7.0, containing proteinase inhibitors and homogenized at 4 °C with 20 strokes of a Dounce tissue grinder (Wheaton, B pestle; 1 ml). Cell extracts were centrifuged at 6600 *g* for 5 min at 4 °C (Beckman Microfuge 11 or Beckman JA20). Mechanical extracts also were subjected to a second centrifugation at 100 000 *g* for 1 h at 4 °C (Beckman, TL 100.2). Extract and pellet fractions were quick-frozen in liquid nitrogen and stored at –70 °C.

Purification of p205 and preparation of antibodies

BHK cells (~10⁸) in 10 ml of 10 mM Tris (pH 7.0) containing proteinase inhibitors were extracted with a Dounce tissue grinder (Wheaton, B pestle, 15 ml) as described above. After centrifugation, the supernatant fraction was dialysed against 10 mM Tris (pH 7.0) and applied to a 1 cm × 12 cm DEAE-cellulose column (DE52, Pierce) previously equilibrated with the same buffer. The unbound fraction was centrifuged at 100 000 *g* (Beckman, TL 100.2) for 1 h at 4 °C, and the supernatant was concentrated in a Micro-ProDicon system (Spectrum, Houston, TX, U.S.A.) with 2 litres of 10 mM Tris (pH 7.0) in the buffer compartment. Samples in reducing SDS-sample buffer (62.5 mM Tris, 2 % SDS, 10 % glycerol, 0.01 % Bromophenol Blue, 5 % mercaptoethanol, pH 6.8) were then fractionated by preparative electrophoresis (Prep Cell; Bio-Rad, Richmond, CA, U.S.A.) according to the manufacturer's instructions. Electrophoresis was carried out in the small Prep Cell column (28 mm internal diam.) with a 6 % -acrylamide running gel (6 cm length). Running buffer consisted of 25 mM Tris, 192 mM glycine and 0.1 % SDS, pH 8.3, and 1 ml fractions were collected.

Polyclonal antibodies were prepared in rabbits with the assistance of the UT Southwestern Animal Resources Center. Samples of p205 purified by preparative electrophoresis (180 µg) were emulsified with 0.5 ml of Hunter's TiterMaxTM R-1 adjuvant (CytRx Corp.) [10] and injected intradermally into two white New Zealand rabbits. Blood was collected 4 weeks later. The IgG fraction of serum was prepared by chromatography on DEAE-cellulose [11]. Protein concentrations were determined by the Bio-Rad DC protein assay according to the manufacturer's instructions.

Abbreviations used: NP-40, Nonidet P-40; DPBS, Dulbecco's phosphate-buffered saline.

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SDS/PAGE and immunoblotting

These were carried out as described previously [5,6]. Samples for electrophoresis were dissolved in reducing SDS-sample buffer (see above) and subjected to SDS/PAGE [12] on 6%-acrylamide minigels (Bio-Rad) or, in Figure 2, a 4–16%-acrylamide gradient slab gel containing a gradient of 3–8 M urea [13]. For immunoblotting, proteins were transferred to nitrocellulose paper (Schleicher and Schuell) and incubated with polyclonal antibodies for 2 h at 22 °C, followed by incubation with alkaline-phosphatase conjugated goat anti-rabbit IgG (Bio-Rad) for 1 h at 22 °C. Detection was accomplished with an alkaline phosphatase conjugate substrate kit, according to the manufacturer's instructions. Protein profiles were determined by Coomassie Blue or silver staining.

Cell blotting

Cell blotting was carried out as described previously [5,6]. Briefly, nitrocellulose strips with transferred protein were blocked with 3% BSA (Miles, Fraction V) in TBS (20 mM Tris base, 0.5 M NaCl, pH 7.5), and then washed with TTBS (TBS+0.05% Tween 20), followed by DPBS. Subsequently, radiolabelled BHK cells were incubated with the strips (see below). The cells had been radiolabelled overnight metabolically with 20 μ Ci/ml [³⁵S]methionine (ICN, ~1100 Ci/mmol) in methionine-deficient Minimal Essential Medium (MEM; Flow Laboratories) supplemented with 10% tryptose phosphate, 10% fetal-bovine serum (Sigma), 1 $\times$ L-glutamine and 20 mM Hepes, harvested by trypsin treatment, and cultured for 3–4 h in suspension in fresh medium without radiolabelled methionine. Each nitrocellulose strip was incubated for 1 h at 37 °C with ~10⁶ cells (3.25 $\times$ 10⁶ c.p.m.) in 0.5 ml of DPBS containing 30 mg/ml BSA (Fraction V). At the end of the incubations, unattached cells were removed by washing with TTBS for 5 min at 100 strokes/min on a reciprocating shaker (New Brunswick R76). The attached cells were fixed with 3% paraformaldehyde and dried for autoradiography. Radioactive bands showed the locations of adhesion-promoting polypeptides. To quantify the results, the autoradiographs were analysed with a Molecular Dynamics densitometer (Sunnyvale, CA, U.S.A.), and band densities were determined by using the 'volume integration' mode.

Amino acid sequence analysis

Sequencing studies were carried out at the UT Southwestern, Howard Hughes Medical Institute Protein Sequencing Facility. Partial amino acid sequence information was acquired by automated Edman degradation with a model 477A sequencer (Applied Biosystems Division of Perkin Elmer, Foster City, CA, U.S.A.) using the manufacturer's standard programming and chemicals. Protein samples (190 μ g, ~918 pmol) were subjected to SDS/PAGE and transferred to Immobilon P (Millipore Corp., Bedford, MA, U.S.A.). The p205 band was located by Coomassie Blue staining, excised, and digested *in situ* with trypsin [14]. Released peptides were purified by reverse-phase chromatography on an Applied Biosystems model 130A HPLC system equipped with a 2.1 mm $\times$ 100 mm Aquapore RP300 column from the same manufacturer. Chromatography was conducted at 50 μ l/min in 0.1% trifluoroacetic acid (TFA) in water, with elution with a 100 min gradient of 0–70% acetonitrile. Peaks were collected and rechromatographed under similar conditions, except with 0.1% (w/v) ammonium acetate in water in place of TFA as the mobile phase.

RESULTS

Adhesion-promoting polypeptides in BHK cell extracts

Figure 1 shows that when BHK cells in suspension were extracted with NP-40 and analysed by cell blotting, fibronectin (FN), p205 and p140 (140 kDa) were the only cell-adhesion-promoting polypeptides detected. Of the three, p205 had the highest activity in the soluble (S) fraction. The possibility that p140 and p205 corresponded to fibronectin fragments similar to those previously detected in wound fluid [5,6] was excluded on the basis of immunoblotting experiments (results not shown).

Adhesion-promoting activity of p140 and p205, like that of fibronectin, was inhibited by adding Arg-Gly-Asp-containing peptides to the cell-blotting assays. Results from several experiments are compiled in Table 1, which shows that 0.5 mg/ml Gly-Arg-Gly-Asp-Ser-Pro inhibited cell adhesion to p205 by 77%,

Figure 1 Cell-blotting analysis of NP-40-extracted and mechanically extracted BHK cells

Suspended BHK cells were extracted by NP-40 or Dounce homogenization, and samples of the extracts were analysed by cell blotting or Coomassie Blue staining. Each lane contained extract equivalent to 10⁶ cells. Samples as indicated were: NP-40 extract, soluble (S) and insoluble (P) fractions; mechanical (Dounce) soluble extract (S-1), and unbound fraction after DEAE-cellulose chromatography (S-2). Samples of plasma fibronectin (pFN), 10 μ g for protein staining and 0.15 μ g for cell blotting, were included as controls. Other details are in the Materials and methods section.

Table 1 Effect of cell-adhesion peptides on cell blotting

Samples of NP-40 extracts of BHK cells (1 $\times$ 10⁶/lane) were subjected to SDS/PAGE, and cell blotting was carried out with peptides GRGDSP (Gly-Arg-Gly-Asp-Ser-Pro) or GRGESp (Gly-Arg-Gly-Glu-Ser-Pro) added at the concentrations indicated. Density of cell binding to p205 was analysed by densitometry using the 'volume integration' mode. Band size of the control samples was 4392 $\pm$ 966 (average $\pm$ S.D.). Results shown are from four separate experiments. Other details are in the Materials and methods section.

Additions (cell blotting)	Inhibition of cell adhesion (%)
Control	0
GRGDSP (0.02 mg/ml)	0
GRGDSP (0.1 mg/ml)	33 $\pm$ 11
GRGDSP (0.5 mg/ml)	77 $\pm$ 11
GRGESp (0.1 mg/ml)	13 $\pm$ 18
GRGESp (0.5 mg/ml)	15 $\pm$ 25

Figure 2 Cell-blotting analysis of substratum-associated material from attached BHK cells

BHK cells (10^6) in 1 ml adhesion medium [MEM (GIBCO) supplemented with 20 mM Hepes, pH 7.2] were incubated in 35 mm culture dishes (Falcon) for 2 h at 37 °C. The dishes were previously coated for 30 min with 10 μ g/ml fibronectin or 10% fetal-bovine serum (GIBCO). At the end of the incubations, attached cells were extracted for 5 min at 22 °C with 0.5% NP-40 in Tris-buffered saline (20 mM Tris, 150 mM NaCl, pH 9.0) supplemented with proteinase inhibitors (0.2 mM phenylmethanesulphonyl fluoride, 1 μ M leupeptin and 1 μ M pepstatin A). Substratum-associated material (SAM) was solubilized in SDS-sample buffer (62.5 mM Tris/HCl, 2% SDS, 10% glycerol, 0.01% Bromophenol Blue, 5% mercaptoethanol, pH 6.8) and analysed by cell blotting. Lane 1, SAM from FBS-coated substratum, + cells; lane 2, SAM from FBS-coated substratum, - cells; lane 3, SAM from fibronectin-coated substratum, + cells; lane 4, SAM from fibronectin-coated substratum, - cells. Other details are in the Materials and methods section.

compared with 15% inhibition by 0.5 mg/ml of the control peptide Gly-Arg-Gly-Asp-Ser-Pro. A large number of non-adhesive but prominent Coomassie-Blue-staining polypeptides in BHK cell extract fractions (Figure 1, 'Protein' lanes) provided additional evidence for the specificity of cell adhesion to p205 and p140.

Occurrence of p205 in substratum-associated material

In other experiments, we tested whether p205 could be detected in the material that remained associated with the substratum after BHK cells previously attached to serum-coated or fibronectin-coated culture dishes were lysed with NP-40. Figure 2 (lanes 1 and 3) shows that substratum-associated material analysed by cell blotting contained both p205 and p140, in addition to several other adhesion-promoting polypeptides. The doublet at 65–75 kDa (lanes 1 and 2) corresponds to vitronectin (VN), the major adhesion protein deposited on culture dishes exposed to 10%-serum-containing medium [15,16]. The 240 kDa component (lanes 1 and 3) corresponds to fibronectin. In the absence of cells, fibronectin could not be detected on 10%-serum-coated dishes (lane 2) (cf. [17]) or on fibronectin-coated dishes. Under the conditions of these experiments, $\sim 1 \mu$ g of fibronectin binds to the dishes, and much of it cannot be solubilized even by SDS [18].

Purification of p205

Approximately equal partitioning of p205 between supernatant (S) and pellet (P) fractions after NP-40 extraction (Figure 1) and retention of p205 in the substratum-associated material after

Figure 3 Fractionation of mechanically extracted BHK cells by preparative electrophoresis

Suspended BHK cells (6.5×10^8) were mechanically extracted, chromatographed through DEAE-cellulose, and the unbound fraction was subjected to preparative electrophoresis. Samples eluted from the gel were analysed by silver staining or cell blotting as indicated. Other details are in the Materials and methods section.

NP-40 extraction of attached cells (Figure 2) suggested that p205 was partially soluble and partially associated with the detergent-insoluble cytoskeletal fraction. After mechanical extraction, p205 also was divided between the soluble (S-1) and particulate fractions (results not shown). As a first step in purification of p205, the mechanical extract was passed through a DEAE-cellulose column, which removed a number of polypeptides as well as most of the fibronectin and p140. The DEAE-unbound fraction (Figure 1, S-2) was then subjected to preparative electrophoresis. Figure 3 presents the protein and biological-activity profiles of fractions recovered after preparative electrophoresis and shows the separation of the p205 polypeptide and adhesion activity from most other proteins.

Polyclonal antibodies were prepared against purified p205, and used for immunoblotting experiments with BHK cell extracts and purified p205. Anti-p205 serum or IgG recognized a major band in BHK cell extracts (Figure 4, lanes 1 and 3) similar to p205 (Figure 4, lane 2). Affinity-purified anti-p205 antibody was prepared by using p205 bound to nitrocellulose as an immuno-adsorbant matrix [19], and was found to react with a single band in BHK cell extracts (Figure 4, lane 5) that corresponded to p205 (Figure 4, lane 4). Anti-p205 antibody also cross-reacted with a similar component in human fibroblasts and human keratinocytes (results not shown). In some immunoblotting experiments, anti-p205 serum and IgG also were observed to bind to ~ 55 kDa polypeptide(s) (Figure 4; see also Figure 5). This reaction was variable, and the ~ 55 kDa peptide(s) may be a myosin degradation product.

Identification of p205 as myosin

Samples of p205 were subjected to partial amino acid sequence analysis, and information was obtained from pools of tryptic peptides separated by reverse-phase HPLC. One of these pools provided data from a single peptide with the sequence Ile-Glu-

Figure 4 Specificity of anti-p205 antiserum by immunoblotting

NP-40 extracts of BHK cells (2.5×10^5) (lanes 1, 3 and 5) or purified p205 (9 µg) (lanes 2 and 4) were subjected to SDS/PAGE and immunoblotted with anti-p205 serum (lane 1), anti-p205 IgG (lanes 2 and 3), or affinity-purified anti-p205 IgG (lanes 4 and 5). Other details are in the Materials and methods section.

Table 2 Full-length myosin type II heavy-chain molecules containing the amino acid sequence Ile-Glu-Trp-Asn-Phe-Ile (ILEWNFI)

Based on IntelliGenetics analysis of the PIR 42 and Swiss-Prot 30 data bases.

Myosin type	Sequence length (residues)	Location of ILEWNFI (residues)
Human, non-muscle type A	1961	498–502
Chick, non-muscle	1959	498–502
Rat, neuronal	1999	497–501
Chick, type B, neuronal	2007	514–519
Frog, non-muscle B isoform	1979	511–516
Chick, gizzard smooth muscle	1972	504–509
Rabbit, smooth muscle	1992	520–525

Trp-Asn-Phe-Ile. An IntelliGenetics search of the PIR 42 and Swiss-Prot 30 protein sequence data banks indicated that this peptide sequence was identical with a segment found in seven different myosin II heavy-chain sequences from non-muscle and smooth-muscle cells, which are summarized in Table 2. This sequence has not been found in other proteins.

Analysis of the other two chromatographic pools, which each contained more than one peptide, identified a mixture of amino acids consistent with the sequences Ile-Ala-Gln-Leu-Glu-Glu-Glu-Leu-Glu-Glu-Glu-Gln-Gly-Asn-Thr and Ala-Leu-Glu-Glu-Ala-Met-Gln-Gln-Lys-Ala-Glu-Leu-Glu. The first of these sequences occurs with 100% similarity in human and chick non-muscle and rat neuronal myosin II heavy chain (Table 2), and with high similarity in other myosin II heavy chains. The second peptide also occurs with higher similarity in myosin II heavy chains compared with other proteins, with 12/13 amino acids identical with those of human non-muscle myosin II heavy chain and 11/13 amino acids identical with chick non-muscle myosin II heavy chain.

The above findings suggested that p205 was the hamster equivalent of non-muscle myosin II heavy chain. This possibility subsequently was confirmed by two additional studies. First, as shown in Figure 5, anti-(bovine trachea myosin II) and anti-p205 antisera reacted with similar bands in immunoblots of BHK cell

Figure 5 Immunoblotting analysis of myosin II and mechanical BHK cell extract

Samples as indicated of pig platelet myosin II (1 µg/lane) or mechanical extract from BHK cells (5×10^5 /lane) were subjected to SDS/PAGE and immunoblotted with anti-(bovine trachea myosin II) serum, anti-p205 IgG, or non-immune serum as indicated. Other details are in the Materials and methods section.

extracts, and anti-p205 was found to react with purified non-muscle myosin II heavy chain. Second, as shown in Figure 6, purified myosin II had cell-adhesion-promoting activity in the cell-blotting assay (lanes 1 and 2) comparable with that observed with BHK cell extracts (lane 3). When purified myosin II head and tail portions were compared, cell-adhesion-promoting activity was associated with the tail domain, but not the head domain (results not shown).

In marked contrast with the adhesion-promoting activity of myosin II heavy chain that was detected by cell blotting, no myosin-dependent adhesion was observed when BHK cells were incubated on culture dishes previously coated for 60 min at 22 °C with solutions of 1–500 µg/ml purified myosin II heavy chain or 10–100 µg/ml myosin II head or tail fragments. In addition,

Figure 6 Cell blotting and immunoblotting analysis of myosin II and NP-40 BHK cell extract

Samples of bovine adrenal-medulla myosin (lanes 1 and 4, 0.5 µg; lanes 2 and 5, 1.0 µg) or NP-40 BHK extract (2.5×10^5 cells) (lanes 3 and 6) were subjected to SDS/PAGE and analysed as indicated by cell blotting or by immunoblotting with anti-(bovine trachea myosin II) serum. Other details are in the Materials and methods section.

myosin-dependent adhesion activity was not observed after NP-40 extraction of BHK cells before SDS treatment. Therefore, the myosin adhesion-promoting domain was normally cryptic and, so far, the only conditions under which we have been able to observe the activity of this domain is after unwinding of the molecule, which occurs under the denaturing conditions of the cell-blotting assay.

DISCUSSION

Using the cell-blotting method, we have discovered a cryptic cell-adhesion domain in myosin II heavy chain. Initially, we observed a 205 kDa cell-adhesion-promoting polypeptide in NP-40 or mechanical extracts of BHK cells. This polypeptide was purified by DEAE-cellulose chromatography followed by preparative SDS/PAGE, and identified as non-muscle myosin II heavy chain, based on sequence analysis and cross-reactivity with anti-(non-muscle myosin heavy chain) antibodies. Moreover, the heavy chain of purified myosin II was found to have cell-adhesion-promoting activity and cross-reacted with anti-p205 antibodies.

The peptide Gly-Arg-Gly-Asp-Ser-Pro specifically inhibited cell adhesion to p205, indicating the involvement of the Arg-Gly-Asp cell-adhesion motif [20]. Moreover, non-muscle myosin II heavy chains from diverse cell types and species contain the Arg-Gly-Asp sequence [21–23]. In the non-muscle myosin II heavy chains identified in Table 2, the amino acid after Arg-Gly-Asp is either Leu or Glu, which distinguishes these Arg-Gly-Asp-containing sequences from those that have been found in the well-characterized adhesion proteins summarized in Table 3.

The location of the Arg-Gly-Asp sequence in myosin is approx. 30 amino acids from the C-terminus, just within the α -helical rod portion of the tail [21], and we found that the myosin adhesion domain was localized to the tail region. This region of the myosin molecule would be cryptic under normal circumstances [24,25], but be likely to unwind and become exposed under the conditions of cell blotting.

The physiological significance of the myosin cryptic cell-adhesion domain is unknown. Based on imaging studies with anti-myosin antibodies [26,27], it has been established that myosin is accessible to the extracellular environment after wounding. Moreover, some myosin proteinase cleavage sites are very close to the Arg-Gly-Asp sequence (e.g. [28]). One speculative possibility, therefore, is that the cell-adhesion domain becomes exposed in the wound environment as a result of proteinase activity.

Regardless of its physiological function, identification of a cryptic cell-adhesion domain in non-muscle myosin has some

interesting implications regarding cell-adhesion recognition sequences. Strong cell-adhesion-promoting activity of non-muscle myosin cannot be explained simply by the presence of an Arg-Gly-Asp sequence, because Arg-Gly-Asp by itself has a rather low affinity for adhesion receptors [29]. Approx. 3 % of all proteins contain Arg-Gly-Asp [30], but few promote cell adhesion even when the Arg-Gly-Asp sequence is exposed as part of the cell-blotting procedure. For instance, although there are a large number of Arg-Gly-Asp-containing proteins in serum and plasma, strong cell binding in the cell-blotting assay occurs only for fibronectin and vitronectin [1,5]. Similarly, myosin heavy chain was the major adhesion-promoting polypeptide in BHK cell extracts.

The ability of the myosin II heavy chain to promote cell adhesion suggests that this molecule contains additional sequence information that works in conjunction with Arg-Gly-Asp to present a strong cell-recognition site. The idea that cell adhesion molecules contain at least two sites that interact co-operatively has been established for fibronectin [31], but non-muscle myosin II heavy chain does not contain any sequences that resemble the Pro-His-Ser-Arg-Asn fibronectin synergy site [32,33]. Moreover, non-muscle myosin II does not contain any of the other well-characterized alternative adhesion domains listed in Table 3, which are found in fibronectin and some other adhesion molecules. Therefore, future studies on non-muscle-myosin-dependent cell adhesion may provide new insights into the nature of protein sequences that can interact synergistically with Arg-Gly-Asp.

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Table 3 Amino acids of adhesion-promoting sequences

Adapted from [34].

Arg-Gly-Asp-related:	
Arg-Gly-Asp-Ser	Fibronectin
Arg-Gly-Asp-Asn	Laminin
Arg-Gly-Asp-Val	Vitronectin
Arg-Gly-Asp-Phe	Fibrinogen
Arg-Gly-Asp-Thr	Collagen type I
Other sequences:	
Leu-Asp-Val	Fibronectin IIICS domain
Arg-Glu-Asp-Val	Fibronectin IIICS domain
Tyr-Ile-Gly-Ser-Arg	Laminin
Asp-Gly-Glu-Ala	Collagen type I

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